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Glomeromycota: two new classes and a new order

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Abstract — Based on concomitant molecular analyses of the ribosomal gene and morphological characteristics, we divide the phylum *Glomeromycota* into three classes: *Glomeromycetes, Archaeosporomycetes,* and *Paraglomeromycetes. Glomeromycetes* are newly organized in three orders: *Glomerales* and *Diversisporales,* both forming typical vesicular arbuscular mycorrhiza with higher plants, and *Gigasporales*, forming arbuscular mycorrhiza without vesicles in the roots but with extra-radical auxiliary cells. Within the phylum, *Archaeosporomycetes* comprise exclusively bimorphic families and genera. The monogeneric *Paraglomeromycetes* species form glomoid spores that typically germinate directly through the spore wall instead through their subtending hyphae.

Key words — evolution, *Gigasporineae*, *Gigasporaceae*, molecular phylogeny, rDNA

Introduction

In 1998, a new fungal class, the *Glomeromycetes*, was proposed, originally within the *Zygomycota*, but subsequently transferred to its own phylum, the *Glomeromycota* C. Walker & A. Schüssler (Schüßler et al. 2001). The phylum is currently represented by 230 arbuscular mycorrhizal (AM) fungi and one fungus living in symbiotic association with algae. The division of *Glomeromycota* into three major clades indicated by the genetic studies of Schüßler et al. (2001) has since been confirmed by other studies (e.g. Redecker & Raab 2006, Msiska & Morton 2009, Oehl et al. 2011a). The first objective of the present study was to confirm the phylogenetic findings and to elucidate the morphological

homologies within and the differences between these three major clades. Consequently, two new classes within the *Glomeromycota* are described in this paper. Our analyses moreover suggest a further division of orders within the major group, the *Glomeromycetes*. We propose to establish a new order for the sporogenous cell-forming AM fungi, which is supported by a series of unique features in addition to the presence of sporogenous cells, such as spore wall and germination characteristics and the presence of extra-radical auxiliary cells instead of intra-radical vesicles (Gerdemann & Trappe 1974).

Material & methods

Morphological analyses

Morphological analyses were performed on type and non-type specimens as described and summarized in a series of recent publications for species of *Glomerales*, *Diversisporales*, *Paraglomerales* (Oehl & Sieverding 2004, Sieverding & Oehl 2006, Oehl et al. 2006, 2008, 2010, 2011a,b, Palenzuela et al. 2008, 2010) and *Archaeosporales* (Spain et al. 2006, Palenzuela et al. 2011). Specimens representing >85% of the known 230 AM fungal species were obtained from public herbaria (OSC, FH, HAC, PDD, Z+ZT, DPP, URM) and collections (Embrapa Agrobiologia (Seropédica, Brazil), International Culture Collection of (Vesicular-)Arbuscular Mycorrhizal Fungi (INVAM)); private collections held by Sieverding, Oehl, Trappe, Błaszkowski, Goto, Herrera, and McGee collection; and the Hall & Abbott (1979) photographic collection. Older (pre-1990) specimens on microscopic slides were mounted in lactophenol; more recent specimens were fixed with polyvinyl alcohol-lactic acid-glycerol (PVLG) or a mixture of PVLG + Melzer's reagent, since 1990 the major fixing media (Brundrett et al. 1994). Newly mounted spores and sporocarps from the collections or from pure cultures were fixed by using the latter two media and sometimes also in a mixture of lactic acid to water at 1:1, in Melzer's reagent, and in water. When available, spores freshly isolated from soils or bait cultures were also mounted and analyzed. Spore morphology terminology follows Oehl et al. (2008, 2011a).

Phylogenetic analyses

Partial sequences of β-tubulin and rRNA (SSU and LSU) genes (obtained from public data bases) were used to reconstruct independent phylogenetic analyses of the *Glomeromycota*. The β-tubulin gene intron sequences were excluded, with only exon regions analysed.

Recent studies (Morton & Msiska 2009) have simultaneously analysed the β-tubulin and rRNA gene sequences. However, as the nucleotide substitution rate in the LSU rRNA clearly differs from that found in the SSU region and β-tubulin gene, we used different nucleotide substitution models in analysing these genes. Furthermore, the rRNA gene fragment (LSU and SSU) sequenced in *Glomeromycota* is represented for > 2400 nucleotide bases whereas there are 600 bases for the β-tubulin sequences, so a comparison is not possible without weighting the data sets correspondingly. Fewer than 50 AM fungal species have been sequenced for both β-tubulin and rRNA genes. We consider it problematic that some sequences were obtained from different isolates

of the same putative species. Some problems with misidentifications that have been reported (see Bago et al. 1998, Lanfranco et al. 2001, Souza et al. 2004, Spain et al. 2006, Sieverding & Oehl 2006) have been repeated in the molecular analyses of such isolates. Thus, we used only partitioned analyses for the different genes and rRNA subunits (β-tubulin, LSU and SSU rDNA) for this study. For consensus analyses, the LSU and SSU trees were considered as only one tree because the sequences of these regions cannot be considered independent data sets. A consensus tree was obtained just for the organization of taxonomic order level or above.

The sequences (all obtained from the National Center for Biotechnology Information-NCBI) were aligned using ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999).

Maximum parsimony (MP) and neighbor joining (NJ) analyses with 1000 bootstrap replications were performed using the Phylogenetic Analysis Using Parsimony (PAUP) program version 4 (Swofford 2003). Bayesian (two runs over 1×10^6 generations with a burnin value of 2500) and maximum likelihood (1000 bootstrap) analyses were executed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5. The nucleotide substitution model was estimated using Topali 2.5 (Milne et al. 2004). Sequences from *Neurospora crassa* Shear & B.O. Dodge, *Boletus edulis* Bull., and *Rhizophydium sphaerotheca* Zopf were used as outgroups for *Glomeromycota*.

Results

General phylogenetics and relations to morphological features

Paraglomerales and *Archaeosporales* have low phylogenetic relationship to *Glomerales* and *Diversisporales* (Figs. 1–3). Species of *Archaeospora, Intraspora, Ambispora,* and *Paraglomus* form extra-radical mycelia and mycorrhizal structures that stain only faintly or not at all in trypan blue (Spain & Miranda 1996, Spain 2003, Spain et al. 2006, Sieverding & Oehl 2006, Walker et al. 2007, Palenzuela et al. 2011). We do not know the reasons for this behaviour. Vesicle formation is rarely reported or might have been based on misinterpretations in *Archaeospora, Intraspora,* and *Paraglomus*. In constrast, in the glomeralean and diversisporalean species, fungal structures stain blue to deep blue with trypan blue and typical vesicular arbuscular mycorrhiza formation is regularly reported (Schüßler et al. 2001). Within the *Glomerales* and *Diversisporales,* only *Gigasporaceae, Scutellosporaceae, Racocetraceae,* and *Dentiscutataceae* apparently do not form intraradical vesicles, but their fungal structures also stain blue to deep blue with trypan blue as in all other glomeralean and diversisporalean families (Bentivenga & Morton 1995, Oehl et al. 2008, 2010). Because such a feature is general and related to the mycorrhiza formation, and as the morphological and physiological characteristics are congruent with the higher phylogenetic clades, there is support for separating the *Archaeosporales* and *Paraglomerales* from the *Glomeromycetes* and for establishing new fungal classes for them in the *Glomeromycota*.

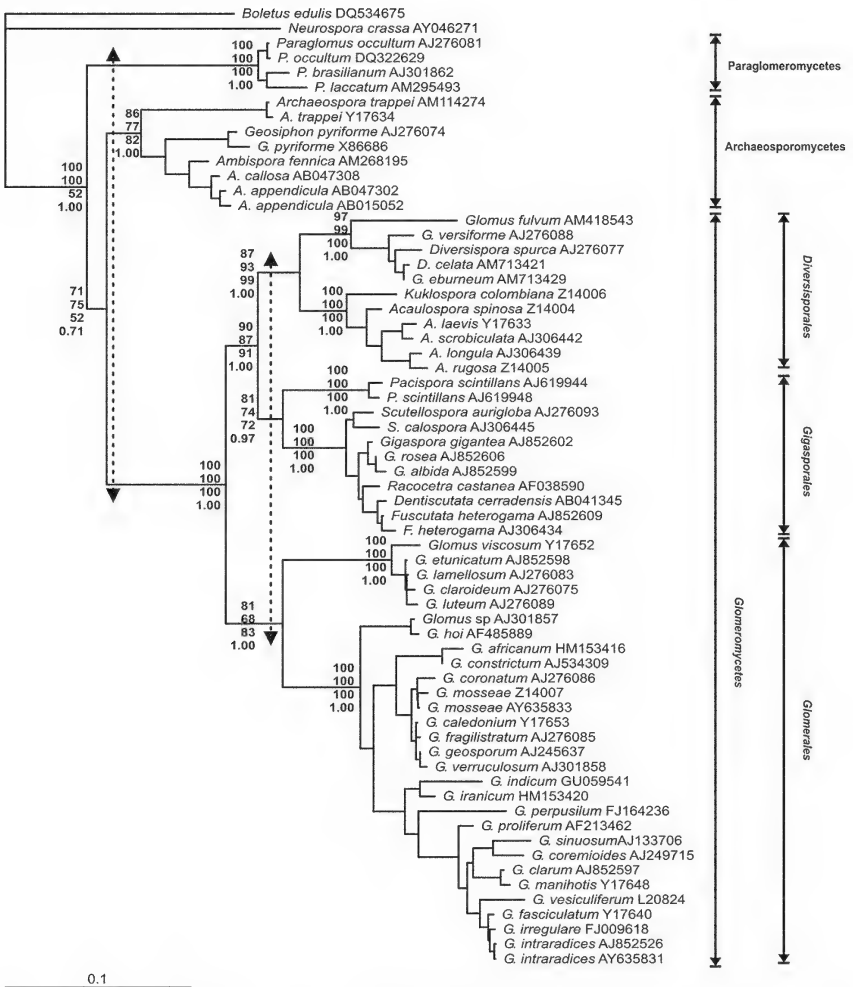
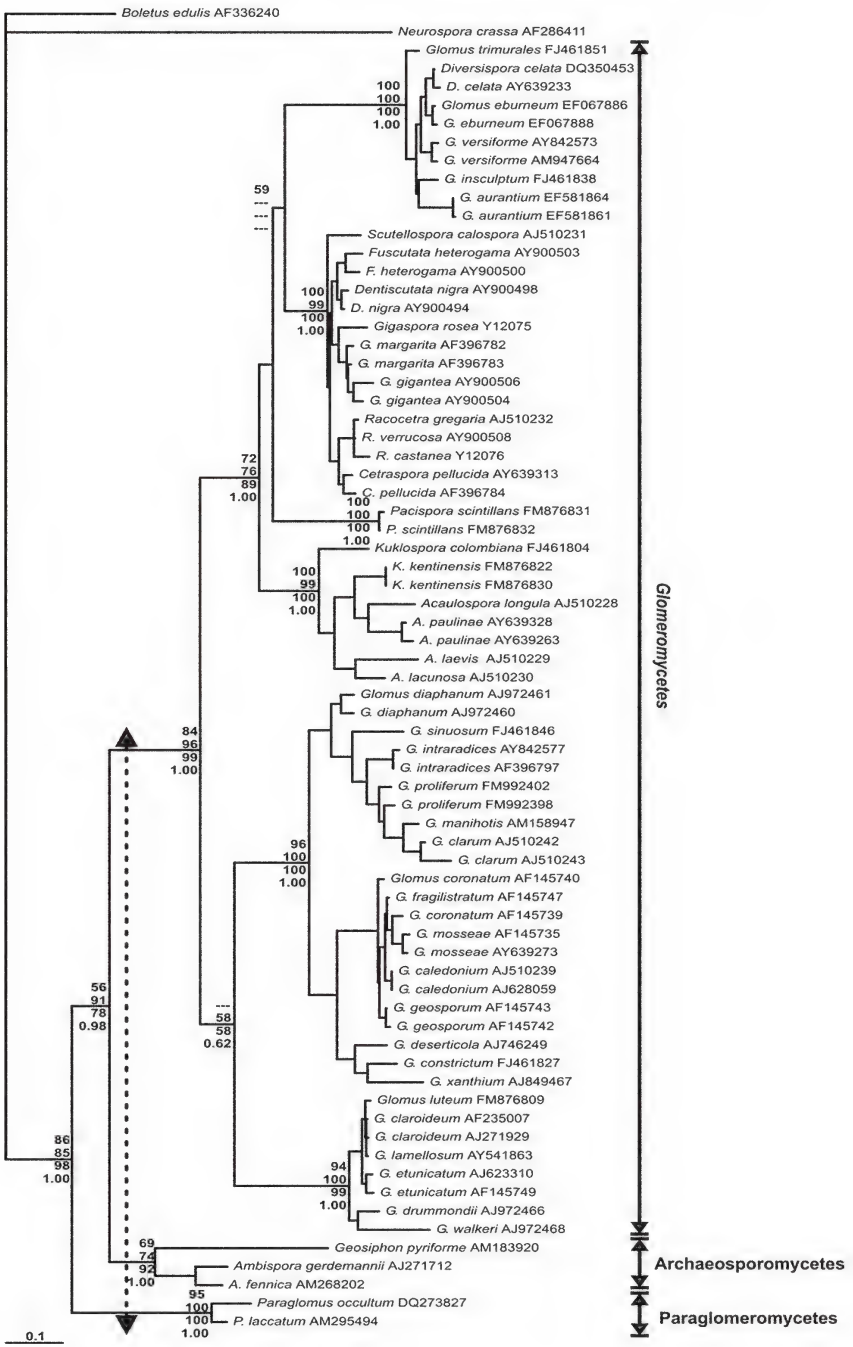


FIG. 1. Phylogenetic reconstruction of the *Glomeromycota* obtained from partial SSU rDNA sequences (~1800 bp). The NJ, ML and Bayesian analyses were performed with GTR+G+I substitution model. Sequences are labeled with their database accession numbers. Support values are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. Only topologies with bootstrap values of at least 50% are shown. (Consistency Index = 0.47; Retention Index = 0.81).

FIG. 2 (right). Phylogenetic reconstruction of the *Glomeromycota* obtained from partial LSU rDNA sequences (~600 bp). The NJ, ML and Bayesian analyses were performed with GTR + G substitution model. Sequences are labeled with their database accession numbers. Support values are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. Only topologies with bootstrap values of at least 50% are shown. (Consistency Index = 0.42; Retention Index = 0.78).


Boletus edulis AF336240
Neurospora crassa AF286411
Glomus trimurales FJ461851
Diversispora celata DQ350453
D. celata AY639233
Glomus eburneum EF067886
G. eburneum EF067888
G. versiforme AY842573
G. versiforme AM947664
G. insculptum FJ461838
G. aurantium EF581864
G. aurantium EF581861
Scutellospora calospora AJ510231
Fuscutata heterogama AY900503
F. heterogama AY900500
Dentiscutata nigra AY900498
D. nigra AY900494
Gigaspora rosea Y12075
G. margarita AF396782
G. margarita AF396783
G. gigantea AY900506
G. gigantea AY900504
Racocetra gregaria AJ510232
R. verrucosa AY900508
R. castanea Y12076
Cetraspora pellucida AY639313
C. pellucida AF396784
Pacispora scintillans FM876831
P. scintillans FM876832
Kuklospora colombiana FJ461804
K. kentinensis FM876822
K. kentinensis FM876830
Acaulospora longula AJ510228
A. paulinae AY639328
A. paulinae AY639263
A. laevis AJ510229
A. lacunosa AJ510230
Glomus diaphanum AJ972461
G. diaphanum AJ972460
G. sinuosum FJ461846
G. intraradices AY842577
G. intraradices AF396797
G. proliferum FM992402
G. proliferum FM992398
G. manihotis AM158947
G. clarum AJ510242
G. clarum AJ510243
Glomus coronatum AF145740
G. fragilistratum AF145747
G. coronatum AF145739
G. mosseae AF145735
G. mosseae AY639273
G. caledonium AJ510239
G. caledonium AJ628059
G. geosporum AF145743
G. geosporum AF145742
G. deserticola AJ746249
G. constrictum FJ461827
G. xanthium AJ849467
Glomus luteum FM876809
G. claroideum AF235007
G. claroideum AJ271929
G. lamellosum AY541863
G. etunicatum AJ623310
G. etunicatum AF145749
G. drummondii AJ972466
G. walkeri AJ972468
Geosiphon pyriforme AM183920
Ambispora gerdemannii AJ271712
A. fennica AM268202
Paraglomus occultum DQ273827
P. laccatum AM295494
0.1
Glomeromycetes
Archaeosporomycetes
Paraglomeromycetes

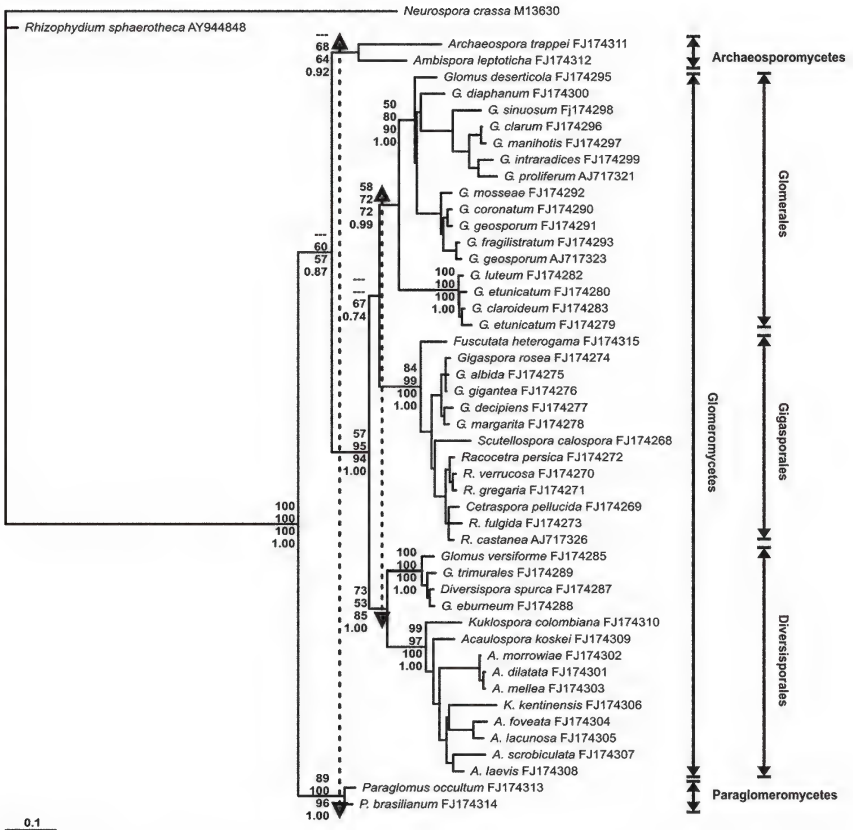


FIG. 3. Phylogenetic reconstruction of the *Glomeromycota* obtained from partial β-tubulin sequences (~600 bp). The NJ, ML and Bayesian analyses were performed with GTR + G + I substitution model. Sequences are labeled with their database accession numbers. Support values are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. Only topologies with bootstrap values of at least 50% are shown. (Consistency Index = 0.37; Retention Index = 0.70).

Archaeosporaceae and *Ambisporaceae* have two different morphs (acaulosporoid & glomoid, or entrophosporoid & glomoid) and form mycorrhiza with plants (Spain et al. 2006; Sieverding & Oehl 2006). *Geosiphonaceae* occur, as far as known, only in association with algae and were until now found with mosses, connected to rhizoid structures. Hence, species of *Archaeosporales* have spores of two different morphs of other AM fungal classes (or order) or are formed by two different groups of organisms. Genetical analyses (see FIGS. 1–3) support the establishment of a new class for these organisms.

Paraglomerales species form glomoid spores with one spore wall as known for species in the *Glomerales*. However, spores germinate directly through the

spore wall (Spain & Miranda 1996, Oehl et al. 2011a), and their extra-radical mycelia and mycorrhizal structures stain only faintly or not at all in trypan blue. Phylogenetic analyses clearly indicate that they are quite distant to species of all other orders in *Glomeromycota*. Hence, we place *Paraglomerales* in a new fungal class.

The *Glomeromycetes* class splits into two major phylogenetic clades: /glomerales and /diversisporales sensu Schüßler et al. (2001) (Figs. 1–3). Spores in these two clades also clearly differ morphologically. Species of *Glomerales* were recently divided in two families (*Glomeraceae* and *Claroideoglomeraceae*) according to spore morphology and phylogenetic analyses (Schüßler & Walker 2010, Oehl et al. 2011a). The *Diversisporales* contain species with highly diverse types of spore formation: ACAULOSPOROID & KUKLOSPOROID (with all spores having three walls and forming on or in the subtending hypha of a sporiferous saccule), GIGASPOROID & SCUTELLOSPOROID (spores forming on sporogenous cells without or with germ shield formation), PACISPOROID (spores with two walls, forming terminally on subtending hyphae, and germinating from the inner wall), DIVERSISPOROID (spores forming terminally on subtending hypha and having one wall that is not entirely continuous with the hyphal mycelia wall; see Oehl et al. 2011a), and ENTROPHOSPOROID & OTOSPOROID (spores with two walls and forming in or on hyphae of sporiferous saccules). The *Diversisporales* currently comprises eight families: *Acaulosporaceae*, *Dentiscutataceae*, *Diversisporaceae*, *Entrophosporaceae*, *Gigasporaceae*, *Racocetraceae*, *Pacisporaceae, Scutellosporaceae*.

Relationships between the families with gigasporoid & scutellosporoid (sensu lato) spore formation and other glomeromycete families are not fully understood. The SSU and LSU rDNA sequence analyses place these families into *Diversisporales*, while the β-tubulin analyses put this group out of *Diversisporales*.

Important morphological features for differentiating species within the *Glomeromycetes* are intraradical vesicle and extra-radical auxiliary cell formation, while species of the *Gigasporineae* form arbuscular mycorrhizas with extraradical auxiliary cells instead of vesicles in roots. All other representatives of the *Glomerales* and *Diversisporales* (excluding *Gigasporineae*) generally form vesicular arbuscular mycorrhizas. Because genetic analyses also clearly indicate sporogeneous cell forming species in a clade separate from the *Diversisporales* and *Glomerales*, we suggest including sporogeneous cell forming species in a new order.

Re-organization of classes in the *Glomeromycota*

Due to the molecular phylogenetic analyses and differences in the type of the arbuscular mycorrhizal structures and spore formation, we divide the *Glomeromycota* into three fungal classes, formally propose the new order

Gigasporales, and emend existing orders and families where necessary. An overview of the new taxonomic organization of the *Glomeromycota* is given in Fig. 4.

Glomeromycetes Caval.-Sm., emend. Oehl, G.A. Silva, B.T. Goto & Sieverd.

Emended description: Glomerospores formed terminally, subterminally or intercalary in hyphae, either in soils or sometimes in roots, either singly, in spore clusters or multiple-spored loose to compact sporocarps, on subtending hyphae (SH), on sporogenous cells, or laterally on or intrahyphally in the stalk of sporiferous saccules, forming arbuscular or vesicular-arbuscular mycorrhiza, with mycorrhizal structures that stain blue to dark blue in trypan blue.

Glomerales J.B. Morton & Benny, emend. Oehl, G.A. Silva, B.T. Goto & Sieverd.

Emended description: Spores formed terminally on or intercalary in hyphae, either in soils or sometimes in roots, either singly, in spore clusters or multiple-spored loose to compact sporocarps, with a single mono-to-multiple layered spore wall. Wall of SH conspicuously continuous with the spore wall and colored the same as or slightly lighter than it or hyaline to subhyaline; SH funnel-shaped, cylindrical or constricted; forming typical vesicular-arbuscular mycorrhiza, with mycorrhizal structures that stain blue to dark blue in trypan blue.

Type species: *Glomus macrocarpum* Tul. & C. Tul. (Clements & Shear 1931)

families included: *Glomeraceae* Piroz. & Dalpé, *Claroideoglomeraceae* C. Walker & A. Schüssler

Genera included: *Funneliformis* C. Walker & A. Schüssler, *Glomus* Tul. & C. Tul., *Claroideoglomus* C. Walker & A. Schüssler, *Septoglomus* Sieverd. et al., *Simiglomus* Sieverd. et al., *Viscospora* Sieverd. et al.

Diversisporales C. Walker & A. Schüssler, emend. Oehl, G.A. Silva & Sieverd.

Emended description: Spore formation acaulosporoid, kuklosporoid, entrophosporoid, otosporoid, or diversisporoid; acaulosporoid and kuklosporoid spores with three walls: multiple layered outer wall, and hyaline middle and inner walls; otosporoid & entrophosporoid spores with two walls: multiple layered outer wall and hyaline inner wall; diversisporoid spores with subtending hyphae (SH) with a conspicuous color change distant to the septum most proximal to the spore base; spores with 1–3 wall layers (SWL1–3), pore rarely open. Forming typical vesicular-arbuscular mycorrhizae, formation of auxiliary cells in root external mycelium unknown.

Type species: *Diversispora spurca* (C.M. Pfeiff. et al.) C. Walker & A. Schüssler

Families included: *Acaulosporaceae* J.B. Morton & Benny, *Diversisporaceae* C. Walker & A. Schüssler, *Entrophosporaceae* Oehl & Sieverd., *Pacisporaceae* C. Walker et al.

genera included: *Acaulospora* Gerd. & Trappe, *Diversispora* C. Walker & A. Schüssler, *Entrophospora* R.N. Ames & R.W. Schneid., *Redeckera* C. Walker & A. Schüssler, *Kuklospora* Oehl & Sieverd., *Otospora* Oehl et al., *Pacispora* Sieverd. & Oehl

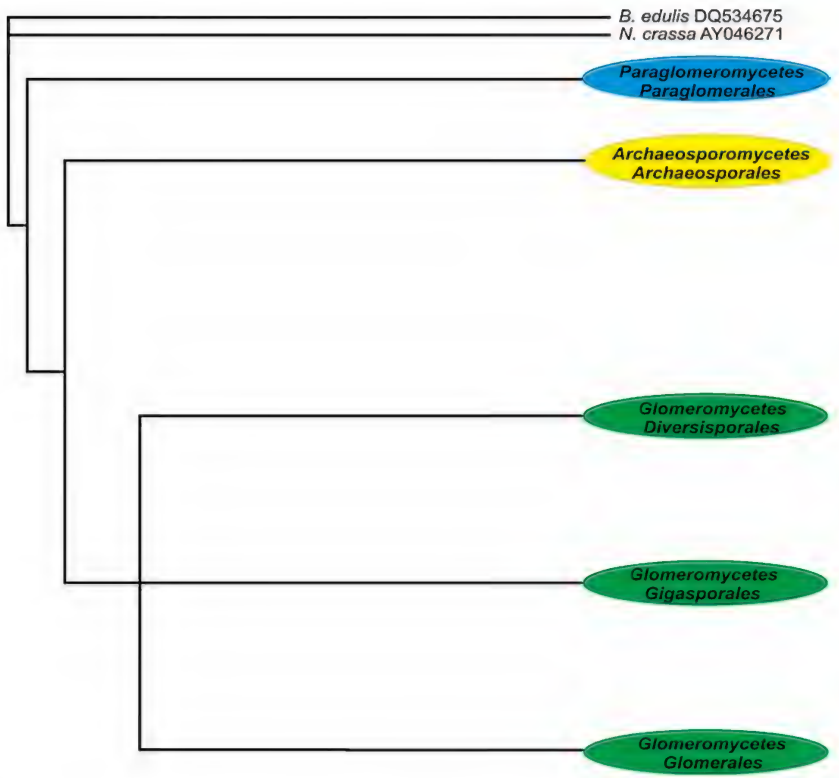


FIG. 4. Cladogram generated by a consensus between the trees obtained from rRNA and β-tubulin genes.

Gigasporales Sieverd., G.A. Silva, B.T. Goto & Oehl, **ord. nov.**

MYCOBANK MB 519688

Sporae terminaliter efformatae anguste adiacetae ad cellulas sporogeneas formans structuras mycorrhizarum arbuscularum et celulas auxiliares; formatio vesiculis ignota.

KEY CHARACTERS: Spore formation gigasporoid and scutellosporoid, i.e. terminally on sporogenous cells, with one to four spore walls; germination from germ warts positioned on the inner surface of single-walled spores or in bi- to multiple-walled spores from germ tube initiations positioned on discrete germination structures (germ shields); arbuscular mycorrhizae and extra-radical auxilliary cells; intra-radical vesicle formation unknown.

TYPE SPECIES: *Gigaspora gigantea* (T.H. Nicolson & Gerd.) Gerd. & Trappe

FAMILIES INCLUDED: *Dentiscutataceae* F.A. Souza et al., *Gigasporaceae* J.B. Morton & Benny, *Racocetraceae* Oehl et al., *Scutellosporaceae* Sieverd. et al.

GENERA INCLUDED: *Cetraspora* Oehl et al., *Dentiscutata* Sieverd. et al., *Fuscutata* Oehl et al., *Gigaspora* Gerd. & Trappe, *Orbispora* Oehl et al., *Quatunica* F.A. Souza et al., *Racocetra* Oehl et al., *Scutellospora* C. Walker & F.E. Sanders

Archaeosporomycetes Sieverd., G.A. Silva, B.T. Goto & Oehl, **cl. nov.**

MycoBank MB 519686

Simbiosis formans inter radices plantarum et fungos vel entre algae et fungi si quod inter radices plantarum et fungos esporae dimorphae; rarum secundum morphum ignotum; arbusculae vel arbuscular et vesiculae formans, estructurae mycorrhizarum non vel pallide tinguntur cum trypan blue.

Key characters: Forms symbiosis between plant roots and fungi, or between algae and fungi; if between plants and fungi, fungal spores with two morphs, rarely only one morph known, and forming vesicular arbuscular or arbuscular mycorrhiza which do not or only faintly stain with trypan blue.

Archaeosporales C. Walker & A. Schüssler, emend. Sieverd., G.A. Silva, B.T. Goto & Oehl

Emended description: Forms simbiosis between plant roots and fungi, or between algae and fungi. If between plants and fungi: fungal species with regularly bi-morphic spore formation: ambi-acaulosporoid & ambi-glomoid, archaeo-acaulosporoid & glomoid, or intra-entrophosporoid & glomoid spore formation; inner walls form de novo; forming arbuscular mycorrhiza; mycelia and mycorrhizal structures do not or only faintly stain in 'trypan blue'.

Type species: *Archaeospora trappei* (R.N. Ames & Linderman) J.B. Morton & D. Redecker

Families included: *Ambisporaceae* C. Walker et al., *Archaeosporaceae* J.B. Morton & D. Redecker, *Geosiphonaceae* Engl. & E. Gilg

genera included: *Ambispora* C. Walker et al. (= *Appendicispora* Spain et al.), *Archaeospora* J.B. Morton & D. Redecker, *Geosiphon* F. Wettst., *Intraspora* Oehl & Sieverd.

Paraglomeromycetes Oehl, G.A. Silva, B.T. Goto & Sieverd., **cl. nov.**

MycoBank MB 519687

Sporae glomoideae; germinatio ex tunica sporae; estructurae mycorrhizae non tinguntur 'trypan blue'; vesiculae ignotae.

Key characters: Spores formed terminally on hyphae; germination directly through spore wall; arbuscular mycorrhiza without or with only faint reaction when exposed to trypan blue; vesicle formation unkown.

Type species: *Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker

family included: *Paraglomeraceae* J.B. Morton & D. Redecker

genus included: *Paraglomus* J.B. Morton & D. Redecker

Discussion

Glomeromycota phylum has three classes and five orders

When Schüßler et al. (2001) described the *Glomeromycota*, they stated that the phylum has one class, the *Glomeromycetes,* established by Cavalier-Smith (1998) for all arbuscular mycorrhizal fungi. Our current molecular analyses (see Figs 1–3) as well as those by the authors of the new phylum and many

other researchers (e.g. Msiska & Morton 2009, Krüger et al. 2009, Gamper et al. 2009, Oehl et al. 2011a) have all consistently demonstrated that no matter which genes are analysed, the *Glomeromycota* is divided into three major monophyletic clades, with a large majority of the known species belonging to the *Glomeromycetes*. We, as Cavalier-Smith (1998) did, think that all species in this class form typical arbuscular mycorrhizal structures in roots. The *Glomeromycetes* can be separated from the other two classes by the staining features of root internal fungal structures. The other two glomeromycotan classes (*Archaeosporomycetes, Paraglomeromycetes*) are new, but were previously recognized as orders (Schüßler et al. 2001). The absent or weak staining of the fungal structures within both new classes suggests a relationship between them. However, the clear genetical differences and the apparent association of two morphologically different organisms (bi-morphic status) in the *Archaeosporomycetes* versus the mono-morphic status of members of *Paraglomeromycetes* species separate these two classes.

Archaeosporales is the only order in *Archaeosporomycetes*. The class contains three clades: the algal symbiont-forming *Geosiphonaceae* and the *Ambisporaceae* and *Archaeosporaceae*. Sieverding & Oehl (2006) and Spain et al. (2006) revised the *Archaeosporaceae* (including the species of *Ambisporaceae*). Genetically, *Geosiphonaceae* appear more closely related to *Ambisporaceae* than *Archaeosporaceae*. However, we have not yet investigated or tried to clarify the relationship of *Geosiphonaceae* with *Ambisporaceae*, leaving that for future research.

Within *Paraglomeromycetes* the single order *Paraglomerales* appears quite homogenous and monophyletic. *Paraglomeromycetes* have glomoid spore formation, and we recognize that interspecific differentiation from members of the *Glomeromycetes* is difficult if one is not familiar with the species-specific characters of the *Paraglomus* spp. Taxonomic separation of *Paraglomeromycetes* from *Glomeromycetes* species has been supported previously only by molecular analyses (Morton & Redecker 2001). However, we found that the root internal mycelium of *Paraglomus* spp. have different staining features than glomoid (*Glomerales*) and diversisporoid (*Diversisporales*) species, and *Paraglomus* spp. obviously lack vesicle formation in the roots, distinguishing them from the vesicular-arbuscular mycorrhiza forming *Glomeromycetes* (Oehl et al. 2011a). Remarkably, while all other glomoid spores germinate through the subtending hyphae, we observed that *Paraglomeromycetes* species generally germinate through the spore wall (Oehl et al. 2011a), an observation needing confirmation for all *Paraglomus* species.

Differentiation of orders in *Glomeromycetes*

The revised class *Glomeromycetes* is divided in three orders: *Glomerales, Diversisporales,* and *Gigasporales*. Within *Diversisporales, Acaulosporaceae* have

particular hyphal attachments, unique spore wall composition, and germination characteristics shared only with ancestral species of *Scutellosporaceae* (Oehl et al. 2008, 2011b). For *Diversisporaceae* (respective former *Glomus* clade C), no attempts or assumptions have been made until very recently (Oehl et al. 2011a) to explain how its species might differ morphologically from the *Glomerales* sensu Oehl et al. (e.g. Schüßler et al. 2001, Walker & Schüßler 2004, Redecker et al. 2007). The spore base of the *Diversisporaceae* is typical and the structural wall layer appears to have formed like an "endospore". In the current study, the morphological differences between glomoid and diversisporoid spores were described, and the family *Glomeraceae* (Pirozynski & Dalpé 1989) was accordingly revised to exclude several former *Glomus* species from the family and transferring them to *Claroideoglomus* or *Viscospora* in the *Claroideoglomeraceae*, to *Diversispora or Redeckera* in the *Diversisporaceae,* or to *Paraglomus* in the *Paraglomeraceae* based on molecular and morphological analyses (Oehl et al. 2011a).

Gigasporales do not include taxa with vesicles and glomoid spore formation. Species of this new order have unique hyphal attachments (sporogenous cells), complex spores, and distinct germination characteristics (Oehl et al. 2008, Goto et al. 2010, 2011). Oehl et al. (2008, 2011b) have recently revised the families, genera and species in *Gigasporales*.

Unsolved problems in the *Glomeromycetes*

Recently, *Pacisporaceae* and *Entrophosporaceae* were included into the *Diversisporales* (Walker & Schüßler 2004, Sieverding & Oehl 2006).

Pacisporaceae fall in two different positions in the SSU and LSU rRNA analyses, and there are no sequences for β-tubulin gene to confirm the phylogenetic relationship between this family and either the gigasporalean or other diversisporalean groups. Since we do not know the real position for *Pacisporaceae*, it was decided to maintain this family within the *Diversisporales*. More studies are required to elucidate the evolutive pathways in *Pacisporaceae* and confirm or correct the actual proposition.

Entrophosporaceae was revised by Sieverding & Oehl (2006). Recent observations suggest a split of the species of *Entrophosporaceae* according to their phylogenetic positions (e.g. see public data bases, Palenzuela et al. 2010). However, further molecular analyses are needed to confirm our observations. Sieverding & Oehl (2006) recently emended the genus *Entrophospora* and its type species.

Last considerations

Taxonomic congruence can be used to determine the true phylogenetic relationships in a group of organisms (Kitching et al. 1998). However this

should be obtained by analyses of independent data sets. When the study involves genes, these should not be genetically linked. Thus, we cannot use LSU and SSU rDNA as two independent data sets to obtain taxonomic congruence.

Diversisporales (sensu Schüßler et al. 2001) form a polyphyletic clade in the β-tubulin analysis and a monophyletic group in the rRNA trees. However, the consensus tree does not establish the former *Diversisporales* as a monophyletic clade in a congruent phylogeny with the two independent data sets (rRNA and β-tubulin genes). Thus, the supports obtained by SSU rDNA and β-tubulin genes sustain *Gigasporales* as a natural taxon. This new order was decribed here to solve the incongruence in *Diversisporales* (sensu Schüßler et al. 2001) and maintain a natural classification in the *Glomeromycota* in the higher taxonomic levels (order and above).

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